

LAB 1

USE OF THE MICROSCOPE

Introduction

Individual microbial cells are usually too small to see with the unaided, “naked” eye. The **binocular compound light microscope** is used to magnify an image and allows us to study the structure of tiny cells. ‘Compound’ means the microscope has two types of lenses (ocular and objective lenses), ‘binocular’ means there are two ocular lenses (allowing better 3-D perception), and ‘light’ means it uses visible light for illuminating the specimen. This instrument is both delicate and expensive, so always be sure to:

- carry the microscope with both hands
- keep microscopes away from the edge of the table
- **use only lens paper to clean the lenses**
- clean all lenses and stage before returning the scope to the cabinet

Part I: Structure and Function of the Compound Microscope

First, be sure you are familiar with the location, operation, and function of the **ocular lenses**, **objective lenses**, **mechanical stage**, **condenser**, **iris diaphragm**, **coarse** and **fine focus**, and **light source** of the microscope (see Figure 1).

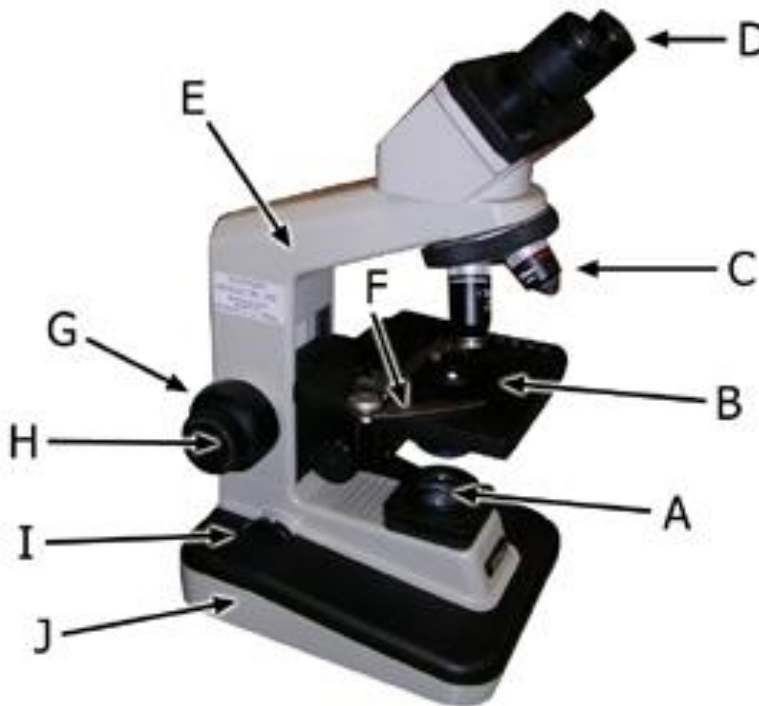


Figure 1. The parts of the compound light microscope.

A. Light Source. Built into the **base (J)**, with a rotating on/off **switch (I)** on the left of the base to control light intensity.

B. Stage. The slide is placed on the stage and clamped in place by the **mechanical arm (F)**. Two knobs below and to the right of the stage allow for precise movement of the slide.

C. Objective Lenses. The rotatable nosepiece or **turret** holds four objective lenses of different magnification: scanning lens (4X magnification), low-power lens (10X), high-dry lens (40X) and oil-immersion lens (100X). Light leaves the objective and goes through the **body tube** to reach the eyepiece.

D. Ocular Lenses (eyepieces). The eyepieces, or lenses you look through. The ocular lens itself provides 10X magnification. You are using a binocular microscope, so called because it has two oculars, one for each eye. Adjust the “interpupillary” distance (between the ocular lenses) until you get a single field of view and not two circles. Closing one eye or using just one ocular will cause eye strain and is not recommended.

The total magnification (i.e., how many times more magnified than the naked eye can see) is determined by multiplying the objective lens power (4, 10, 40 or 100X) by the ocular lens power (10X). The various lens combinations are outlined in the table below:

Table 1. The objective lenses and their relationship to the total amount of magnification.

Objective lens	Ocular lens	Total Magnification
scanning lens 4X	10X	40X
low-power lens 10X	10X	100X
high-dry lens 40X	10X	400X
oil-immersion lens 100X	10X	1000X

E. Arm. Connects the **base (J)** to the **ocular lenses (D)**.

F. Mechanical Arm. The mechanical arm, or stage clip, clamps the slide in place on the stage.

G. Coarse Focus Knob. This larger, outer knob allows for rapid focusing of the specimen.

H. Fine Focus Knob. This smaller, inner knob allows precise focus adjustment.

I. Light Switch. Located on the left of the **base (J)**, rotates on/off to control light intensity.

J. Base. Stabilizes the microscope and contains the **light switch (I)** and the **focus knobs (G, H)**.

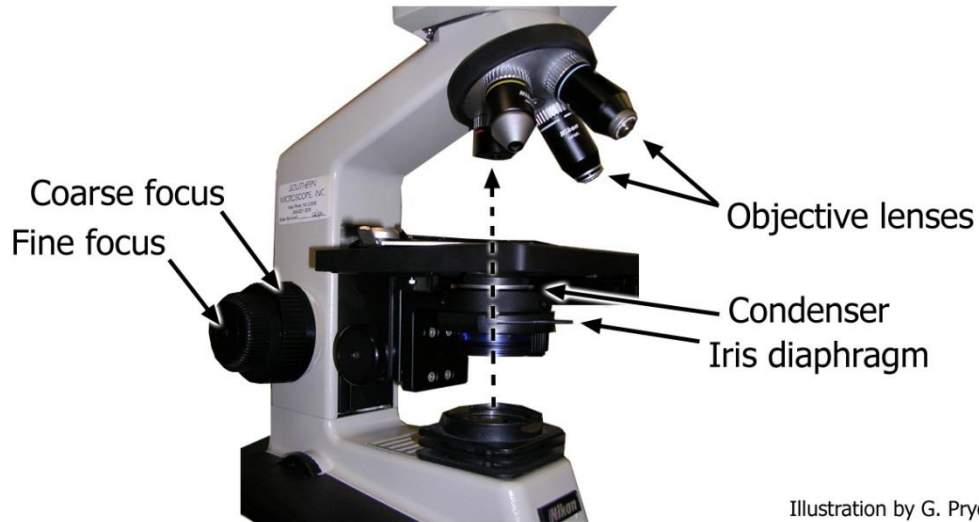


Illustration by G. Pryor

**Figure 2. Another view of the compound light microscope.
The path of light is indicated by the dashed-line arrow.**

Iris Diaphragm. Attached to the bottom of the condenser, this controls the diameter of the light path, and thus the amount of light entering the objective lenses. A lever projects outward and allows adjustment of the iris diaphragm, to provide the best view with the optimum contrast and lighting.

Condenser. This is an additional lens that focuses the light onto the specimen on the slide. A knob adjusts the condenser up and down, but you should not need to adjust it.

You should understand the path of light through the parts of the microscope, and how to adjust the amount of light entering the lenses. Also know how to change magnification, contrast, and focus.

The overall path of light through the compound light microscope is thus:

Light Source → Iris Diaphragm → Condenser → Slide/Specimen → Objective Lens → Ocular Lens → Your Eyes

Part II: Using the Microscope

You will use the compound microscope to examine a variety of ‘prepared’ (dead, preserved, and stained) microscopic cells.

Procedures

Obtain:

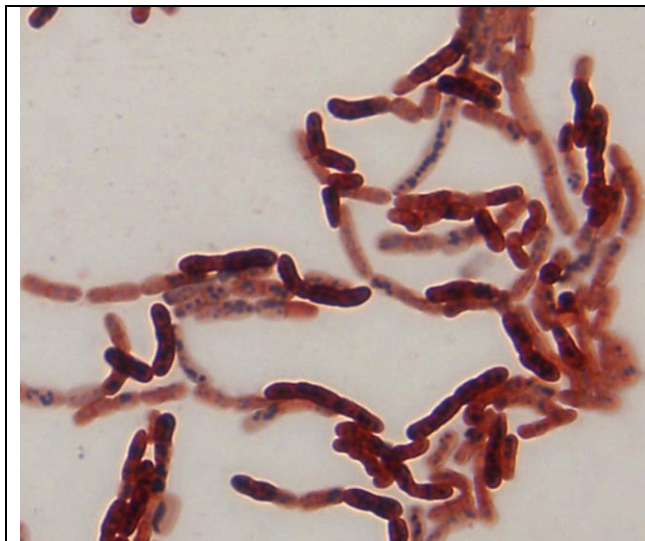
- prepared slides of the following specimens:

Bacteria	Cell shape/morphology
<i>Bacillus megaterium</i>	rod-shaped bacteria (bacilli)
<i>Streptococcus pneumoniae</i>	spherical-shaped bacteria (cocci),
<i>Treponema pallidum</i>	spiral-shaped bacteria (spirilla),

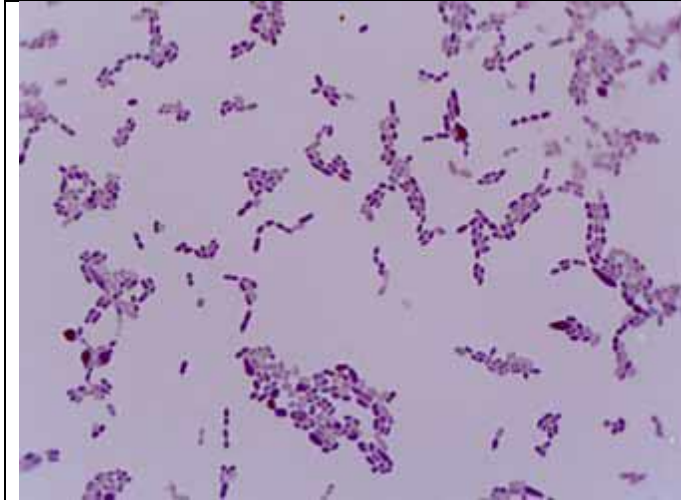
Rotate the light switch toward you to turn on the lamp. The intensity should be increased as you go to higher power objectives. The iris diaphragm should also be adjusted for each objective to give a uniform, bright view with maximum clarity.

Using the coarse focus knob, lower the stage away from the objective lens and then place a slide on the stage. Locate the specimen on the slide using a low magnification (4X or 10X objective). Get the specimen in focus, even if it just looks like a “blur.” Next, change objective lenses to the next higher magnification. Get it in focus and repeat this process until you are looking at the specimen with the 40X objective. Do not change the focus before the next step!

Bacteria are so small they need to be viewed at 1000X, or under oil immersion. To do this, rotate the 40X objective lens away from the slide and place a drop of oil on the slide where the light passes through the specimen. Rotate the 100X lens into the oil. Use the fine focus knob to find the bacteria under oil immersion. This might take a lot of focusing with the fine focus knob!



Bacillus megaterium bacteria, a common soil microbe (1000X).



Streptococcus pneumoniae, a cause of pneumonia, a respiratory disease (1000X).



Treponema pallidum, the cause of syphilis, a sexually-transmitted disease (1000X).

Part III: Wet Mount of Yeast Cells

Now that you have gained some experience with prepared slides, you will next prepare a fresh **wet mount** slide of the yeast *Saccharomyces cerevisiae*. This is a unicellular fungus (NOT bacteria!) that is commonly used when making bread and alcoholic beverages. The yeast *Saccharomyces* is transparent and barely visible under the microscope without staining. It will look much better after you stain it!

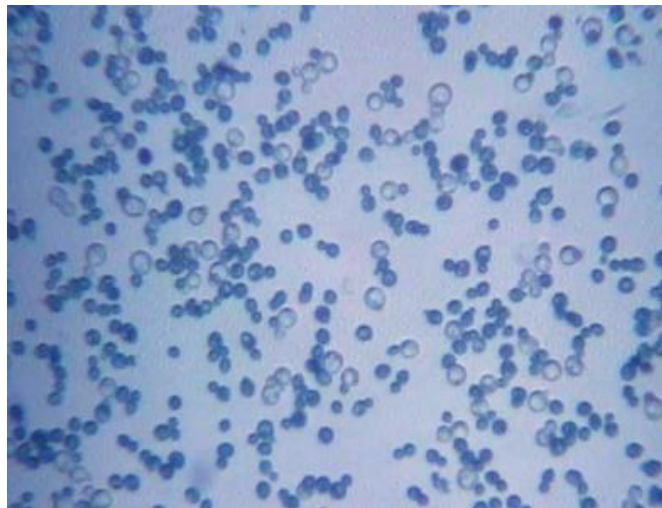
Procedures

Obtain

- yeast suspension
- new, cleaned slide
- coverslip
- methylene blue stain

To make an unstained wet mount, add a drop of the yeast suspension to a slide, then cover the drop with a coverslip. Get the cells into focus. The cells will look like small spheres. After viewing with the 4X objective, rotate the turret until the 10X objective clicks into place. Because the yeast cells are relatively large (compared to bacteria), you will only need to go up to the 40X objective; the oil immersion lens is not needed in this case.

To make a stained wet mount, add a drop of methylene blue to the slide you have already made, next to the coverslip (NOT on top of the coverslip or under the coverslip!). Gently move the coverslip so that the edge of the coverslip touches the dye. Some of the dye should get 'sucked' under the coverslip. Next, using a small piece of paper towel, absorb the excess dye into the paper towel away from the coverslip. Some of the dye will remain under the coverslip. This is the area you should look at under the microscope. The yeast cells will look like small blue spheres.



Saccharomyces cerevisiae yeast stained with methylene blue (400X).